Biochimica et Biophysica Acta, 679 (1982) 376–383
Elsevier Biomedical Press

BBA 41038

EVIDENCE FOR TWO DIFFERENT HERBICIDE-BINDING PROTEINS AT THE REDUCING SIDE OF PHOTOSYSTEM II

WALTER OETTMEIER, KLAUS MASSON and UDO JOHANNINGMEIER

Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität, D-4630 Bochum 1 (F.R.G.)

(Received May 27th, 1981)
(Revised manuscript received August 31st, 1981)

Key words: Photosystem II; Photoaffinity labelling; Herbicide-binding protein

The binding behaviour at the thylakoid membrane of the radioactively labelled phenolic inhibitors 2-iodo-4-nitro-6-[2′,3′-^{3}H]isobutylphenol and 3,5-diiodo-4-hydroxy[U-^{14}C]benzonitrile (ioxynil) has been studied. As judged from displacement experiments with other herbicides, phenolic herbicides and herbicides as represented best by 3-(3,4-dichlorophenyl)-1,1-dimethylurea have different binding sites at the reducing side of Photosystem II. The binding parameters of phenolic herbicides are not, or only slightly, affected by trypsin treatment of chloroplasts. In chloroplasts, besides free pigments, lipids, and the light-harvesting chlorophyll *a/b* protein complex, a protein of molecular weight 41 000 is radioactively labelled by the photoaffinity lable 4-nitro-2-azido-6-[2′,3′-^{3}H]isobutylphenol. The amount of radioactivity bound to the 41 kDa protein is diminished if chloroplasts are incubated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea prior to addition of the photoaffinity label, but not if the 2,4-dinitrophenyl ether of 2-iodo-4-nitrothymol is used instead. These two compounds are characteristic representatives of inhibitiors acting at the reducing or the oxidizing site of plastoquinone, respectively. Based on these results, a model for two different herbicide-binding proteins at the reducing side of Photosystem II is presented.

Introduction

The target of herbicides which act as inhibitors of the photosynthetic electron-transport chain is exclusively located at the reducing side of PS II. According to the present view, the mechanism of action of these herbicides is believed to be due to a reversible and non-covalent binding to a protein called either B [1] or R [2]. This binding leads to a conformational change of the protein, which in consequence lowers the midpoint potential of a special plastoquinone bound to this protein [2], and thus photosynthetic electron flow is inhibited.

So far, two different classes of PS II herbicides have been recognized. The first class is comprised of a variety of chemically different compounds which, however, all share a common structural element, N-C=X, where X signifies N or O. DCMU is the prototype of herbicides of this class. The second class consists entirely of phenols which in addition to the aromatic hydroxyl group bear nitro and/or halogen and/or nitrile substituents. They will be termed phenolic herbicides (for a recent review see Ref. 3). Phenolic herbicides are not only inhibitors of photosynthetic electron transport, but also uncouplers of photophosphorylation ('inhibitory uncouplers' [4]).

For both classes of herbicides, despite the lack of the common structural element mentioned above for

Abbreviations: atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine; azido-atrazine, 2-azido-4-ethylamino-6-isopropyl-amino-*S*-triazine; Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP-INT, 2.4-dinitrophenyl ether of 2-iodo-4-nitrothymol; i-dinoseb, 2,4-dinitro-6-isobutylphenol; ioxynil, 3,5-diiodo-4-hydroxybenzonitrile; PS II, Photosystem II; SDS, sodium dodecyl sulphate.

phenolic herbicides, an identical inhibition site in photosynthetic electron flow has been assumed [3,5–11].

In this paper, for which preliminary reports have been given [12–14], we want to present evidence that the binding and inhibition sites for herbicides related to DCMU and phenolic herbicides are located at two different protein components, although there exists a close spatial arrangement and strong interaction between each other. This is concluded from competition experiments with radioactively labelled phenolic herbicides and by photoaffinity labelling of the thylakoid membrane. A phenolic azide derivative predominantly labels a protein of molecular weight approx. 41 000. This result has to be compared with recent findings obtained with the photoaffinity label azido-atrazine which is reported to mark a protein of molecular weight approx. 32 000–34 000 [15–18].

Materials and Methods

Synthesis and source of chemicals

2-Iodo-4-nitro-6-isobutylphenol. 0.96 g (4 mmol) of 2,4-dinitro-6-isobutylphenol [19] in 8 ml H_2O and 8 ml conc. NH_3 were refluxed with 4 ml 40% $(NH_4)_2S$ for 45 min. After cooling to room temperature, conc. HCl was added until the colour of the reaction mixture changed to pale yellow. After addition of another 3 ml of conc. HCl refluxing was continued for 15 min. After cooling to room temperature the mixture was filtered, and the filtrate extracted five times with ether. The ether phase was dried over $MgSO_4$ and evaporated under vacuum. The residue was taken up in 16 ml of 35% fluoroboric acid and cooled in an ice bath. A solution of 0.55 g $NaNO_2$ in 2.4 ml H_2O was added dropwise over a period of 15 min under vigorous stirring and the temperature of the reaction mixture was maintained at 0°C. After an additional 15 min, excess $NaNO_2$ was destroyed by addition of 0.48 g urea. Then 6.0 g KI in 15 ml H_2O were added and the reaction mixture allowed to stand for 24 h. It was extracted three times with ether, elementary iodine destroyed by $Na_2S_2O_3$, the ether phase dried over $MgSO_4$ and evaporated under vacuum. The residue was taken up in a small amount of benzene and chromatographed first on aluminium oxide and then on silica gel (E. Merck, Darmstadt, F.R.G., columns 3.5 × 15 cm, eluent benzene). Yield 0.23 g (18%); m.p. 51°C. Analysis: Calc. (%) for $C_{10}H_{12}INO_3$ (321.1): C, 37.40; H, 3.77; N, 4.36; I, 39.5. Found (%): C, 37.27; H, 3.79; N, 4.35; I, 39.9. Absorption spectrum (10 mM phosphate buffer, pH 8.0): 278 nm ($\epsilon = 5300\ M^{-1} \cdot cm^{-1}$), 423 nm ($\epsilon = 19\,200\ M^{-1} \cdot cm^{-1}$).

2-Iodo-4-nitro-6-[2′,3′-^{3}H]isobutylphenol. 39 mg (162 μmol) 2,4-dinitro-6-[2′,3′-^{3}H]isobutylphenol [19] (spec. act. 490 mCi/mmol) were reacted with the appropriate amounts of reagents under the same conditions as described above. The final reaction product was chromatographed in aliquots of 70 μl on silica gel precoated plastic sheets (Polygram SIL G/UV$_{254}$, thickness 0.25 mm; Macherey-Nagel and Co. GmbH, Düren, F.R.G.) with benzene as the solvent. The zone corresponding to 2-iodo-4-nitro-6-[2′,3′-^{3}H]isobutylphenol ($R_f = 0.69$) was cut out and eluted with methanol. The concentration of the phenol was determined from its absorption maximum at 423 nm. 2-Iodo-4-nitro-6-[2′,3′-^{3}H]isobutylphenol was obtained in a radiochemical yield of 9.6% and with a specific activity of 333 mCi/mmol.

The synthesis of 4-nitro-2-azido-6-[2′,3′-^{3}H]isobutylphenol has been described recently [12]. [*ring*-^{14}C]Ioxynil (spec. act. 12.5 mCi/mmol) was a generous gift from Dr. R.H. Hewett, May and Baker Ltd., Ongar, U.K.

Biochemical methods

Chloroplasts from spinach were prepared according to Ref. 20 and for the labelling experiments as described in Ref. 12. I_{50} values in photosynthetic ferricyanide reduction were evaluated as reported by Tischer and Strotmann [21]. They were extrapolated graphically to zero chlorophyll concentration. Trypsin treatment of chloroplasts was performed according to Ref. 22. Binding of radioactivity labelled inhibitor to chloroplasts was determined as described recently (Method B [19]). The labelling procedure for 4-nitro-2-azido-6-[2′,3′-^{3}H]isobutylphenol, SDS solubilisation of chloroplasts, and conditions for polyacrylamide gel electrophoresis have been reported elsewhere [12]. For estimation of molecular weights (in parentheses) the following proteins served as markers: cytochrome *c* (12 500), chymotrypsinogen A (25 000), egg albumin (45 000) and bovine serum albumin (68 000).

Results and Discussion

Non-competitive binding between DCMU and phenolic herbicides

A comparison of the binding behaviour of DCMU and phenolic herbicides in isolated chloroplasts is worthy of special interest. We have recently reported on the synthesis and thylakoid membrane binding properties of radioactively labelled i-dinoseb, an isomer of the well known herbicide dinoseb [19]. The specific (high affinity) binding of i-dinoseb to the thylakoid membrane, however, is masked by a high degree of unspecific (low affinity) binding. Furthermore, inhibition and binding constants differ by a factor of 40 [19]. In 2-iodo-4-nitro-6-[2′,3′-^{3}H]isobutylphenol we have synthesized a representative of a phenolic herbicide which belongs to a class of very efficient inhibitors of photosynthetic electron transport [11]. In addition, by courtesy of May and Baker, Ltd., U.K., [^{14}C]ioxynil has been made available to us.

The pI_{50} values and specific binding data (the unspecific binding has been subtracted) for both phenolic herbicides are summarized in Table I. As can be seen, pI_{50} values for both compounds, extrapolated to zero chlorophyll concentration, exceed that of i-dinoseb (pI_{50} 5.45 [19]), by more than two orders of magnitude. The pK_b and extrapolated pI_{50} values are in good agreement with each other for ioxynil. This indicates a 1 : 1 relationship between binding and inhibition [21]. The small difference between these two values for 2-iodo-4-nitro-6-isobutylphenol might imply additional binding at a site not related to inhibition of electron transport. For 2-iodo-4-nitro-6-isobutylphenol and ioxynil the number of binding sites corresponds to one molecule inhibitor per 418 or 540 Chl molecules, respectively, i.e., about one molecule inhibitor per electron-transport chain.

For both phenolic herbicides, binding to isolated chloroplasts in the presence of other inhibitors has been investigated. In Figs. 1 and 2 double-reciprocal plots (for the theory see Ref. 21) for specific binding of 2-iodo-4-nitro-6-isobutylphenol and ioxynil, respectively, in the presence of increasing concentrations of DCMU are demonstrated. As can be seen in both cases, the regression lines share an identical abscissa intercept, which is indicative of identical binding constants for the control and at increasing DCMU concentrations. On the other hand, the ordinate intercepts, which are a measure of the number of binding sites, are different in all cases. According to classical enzyme kinetics, which are applicable for herbicide binding [21], this is a clear indication of a non-competitive interaction between phenolic herbicides and DCMU.

In a similar experiment, the binding behaviour of ioxynil in the presence of increasing amounts of 2-iodo-4-nitro-6-isobutylphenol has been investigated.

TABLE I

pI_{50} VALUES AND BINDING PARAMETERS FOR THE PHENOLIC HERBICIDES 2-IODO-4-NITRO-6-ISOBUTYLPHENOL AND IOXYNIL IN CONTROL AND TRYPSIN-TREATED CHLOROPLASTS

Compound	pI_{50} (uncoupled ferricyanide reduction, extrapolated to zero Chl concentration)	Binding constant K_b (μM)	pK_b	Number of binding sites (nmol/mg Chl)	Number of Chl molecules per molecule inhibitor
Control					
2-Iodo-4-nitro-6-isobutylphenol	7.52	0.010	7.99	2.66	418
Ioxynil	7.62	0.013	7.90	2.06	540
Trypsin treated (10 min)					
2-Iodo-4-nitro-6-isobutylphenol	–	0.009	8.04	2.73	406
Ioxynil	–	0.008	8.09	1.32	840

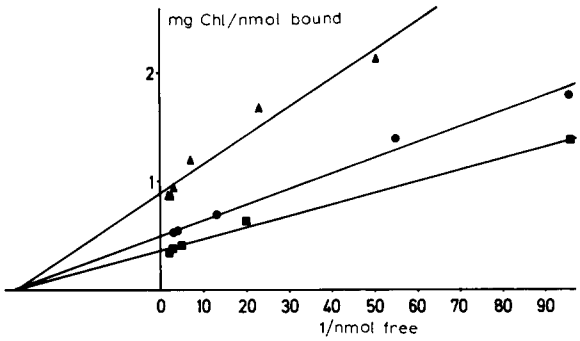

Fig. 1. Double-reciprocal plots for specific binding of 2-iodo-4-nitro-6-[2',3'-^{3}H]isobutylphenol to chloroplasts in the presence of DCMU. ■———■, control; ●———●, plus 0.05 nmol DCMU; ▲———▲, plus 0.2 nmol DCMU. The concentration of chloroplasts corresponded to 100 μg Chl.

In this case, the abscissa intercepts of the regression lines are different, but the ordinate intercepts are identical (Fig. 3). The same experiment as performed for binding of 2-iodo-4-nitro-6-isobutylphenol in the presence of increasing concentrations of ioxynil (results not shown) yields the same picture. This interaction clearly has to be considered as a competitive one. From these results it has to be concluded that DCMU, and to generalize, compounds functionally related to DCMU, and phenolic herbicides have different binding sites.

Treatment of chloroplasts with the proteolytic enzyme trypsin is known to diminish or even abolish the high-affinity binding of DCMU-type herbicides [22,23]. In contrast, the binding constants for

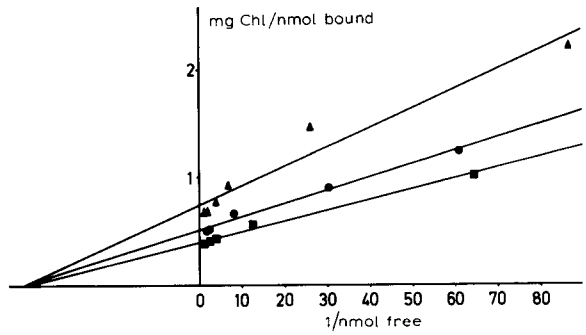

Fig. 2. Double-reciprocal plots for specific binding of [^{14}C]-ioxynil to chloroplasts in the presence of DCMU. ■———■, control, ●———●, plus 0.1 nmol DCMU; ▲———▲, plus 0.2 nmol DCMU. Chl concentration same as in Fig. 1.

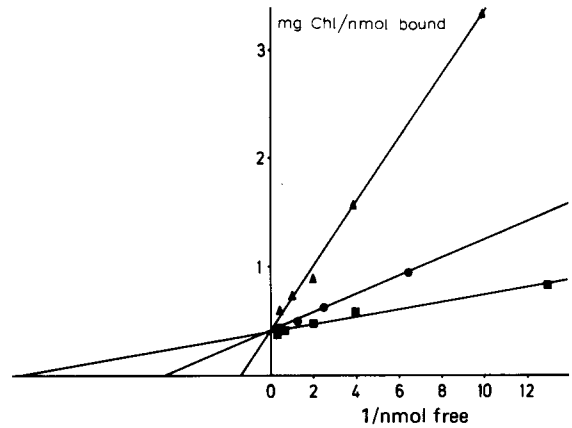

Fig. 3. Double-reciprocal plots for specific binding of [^{14}C]-ioxynil to chloroplasts in the presence of 2-iodo-4-nitro-6-isobutylphenol. ■———■, control; ●———●, plus 0.2 nmol and ▲———▲, plus 0.2 nmol 2-iodo-4-nitro-6-isobutylphenol. Chl concentration same as in Fig. 1.

2-iodo-4-nitro-6-isobutylphenol or ioxynil are not affected by trypsin treatment. There is, however, a small decrease in the number of binding sites for ioxynil after trypsin digestion (Table I).

Photoaffinity labelling of the binding protein for phenolic herbicides

By reaction at the *o*-nitro group, the phenolic herbicide i-dinoseb could be converted into the photoaffinity label 4-nitro-2-azido-6-[2',3'-^{3}H]isobutylphenol [12]. For this compound, a pI_{50} value of 5.70 in uncoupled photosynthetic NADP reduction, a binding constant of 0.19 μM and a number of binding sites of 4.03 nmol/mg Chl, corresponding to one molecule azide per 280 Chl molecules were reported [12]. By illumination with white light at high light intensity the phenylazide is linked covalently and thus irreversibly to the thylakoid membrane. A direct proof for this irreversible binding is demonstrated in Fig. 4. If chloroplasts are incubated in strict darkness with 4-nitro-2-azido-6-[2',3'-^{3}H]isobutylphenol, allowed to equilibrate and then increasing concentrations of i-dinoseb added, i-dinoseb is capable of displacing the phenylazide from the membrane. At a concentration of 10^{-5} M i-dinoseb, more than 60% of the originally bound azide is removed from the thylakoid membrane (Fig. 4). In contrast, if chloroplasts are illuminated in the presence of the azide for 15 min, and then i-dinoseb is added, two important dif-

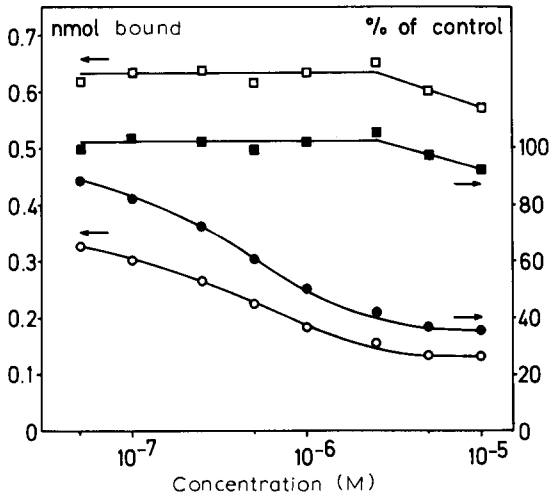

Fig. 4. Displacement of 4-nitro-2-azido-6-[2′,3′-^{3}H]isobutylphenol (1 nmol = $5 \cdot 10^{-7}$ M) from the thylakoid membrane by i-dinoseb before (o———o) and after (□———□) 15 min illumination with white light. The closed symbols indicate the % of control binding. Chl concentration same as in Fig. 1.

ferences with regard to the dark control can be noted. Firstly, the amount of radioactivity bound is about twice as much as compared to the dark control. This is due to the fact that the nitrene generated by illumination binds not only to the specific binding sites, but also to other sites not correlated with the inhibition site. Secondly, the reaction product derived from the azide cannot be displaced any more from the thylakoid membrane by i-dinoseb. Even at a concentration of 10^{-5} M i-dinoseb, less than 10% of the originally present compound is removed from the membrane (Fig. 4). This is a clear indication of covalent and irreversible binding of the azide to the thylakoid membrane, or as expressed in more exact terms, of the reaction products of the corresponding nitrene.

Chloroplasts labelled with 4-nitro-2-azido-6-[2′,3′-^{3}H]isobutylphenol were solubilized by SDS treatment and chloroplast proteins subsequently separated by gradient polyacrylamide gel electrophoresis. The resulting labelling pattern is shown in Fig. 5. It is very similar to that reported previously [12]. The peak heights, however, and the resolution of the single compounds may differ somewhat due to the technique of slicing the gel into small 2-mm pieces which subsequently are oxidized and assayed for radioactivity by liquid scintillation counting. Like i-dinoseb [19], the phenylazide exhibits a large amount of unspecific binding. Consequently, the highest amount of radioactivity is found to be associated with low molecular weight compounds, presumably lipids and pigments (right-hand side, Fig. 5). Four chloroplast proteins are labelled to different extents. The highest amount of radioactivity is found in a protein of molecular weight approx. 41 000. Furthermore, three other proteins with the following molecular weights are labelled to a minor extent: 32 000, 25 000 and 17 000. The protein of molecular weight 25 000, as judged from the highest protein content of all chloroplast proteins (trace for absorbance in Fig. 5), corresponds to the light-harvesting chorophyll *a/b* protein complex [24]. As we have previously reported [12],

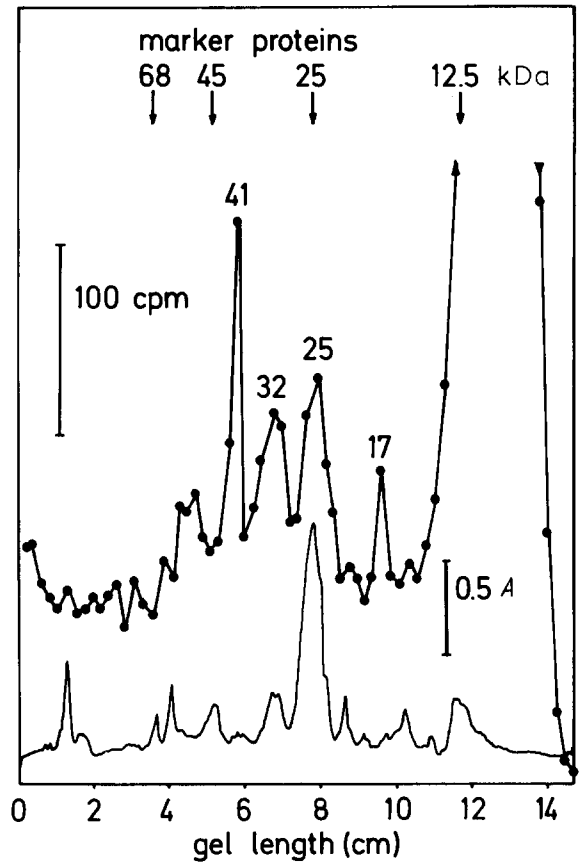

Fig. 5. Scan for radioactivity (upper trace) and absorbance at 620 nm (lower trace) of an SDS-polyacrylamide gel (11–15%) of chloroplasts labelled with 4-nitro-2-aziodo-6-[2′,3′-^{3}H]-isobutylphenol (8 nmol/mg Chl).

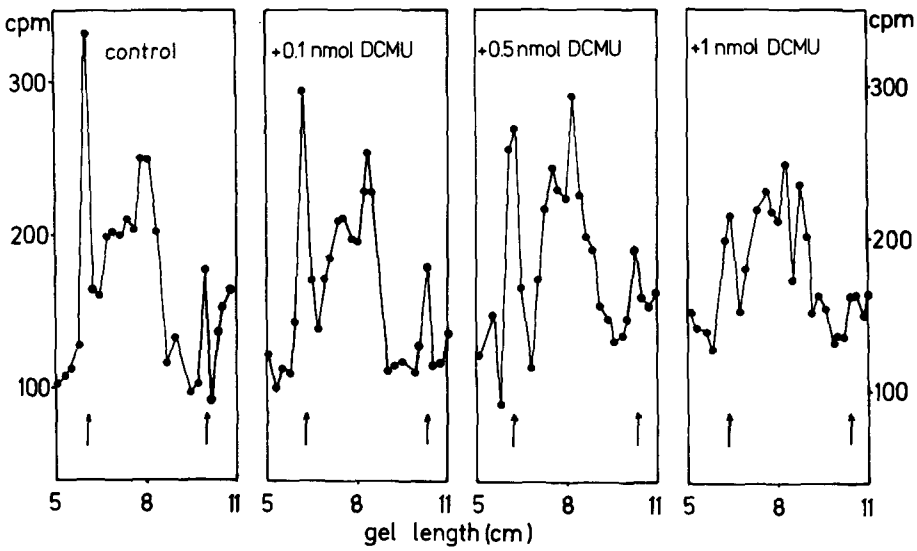

Fig. 6. Scan for radioactivity of a part of the SDS-polyacrylamide gels (11–15%) of chloroplasts labelled with 4-nitro-2-azido-6-[2',3'-^{3}H]isobutylphenol (8 nmol/mg Chl) in the presence of DCMU.

radioactivity in the 41 kDa protein and in addition in the 17 kDa protein is not found, if chloroplasts are incubated prior to the addition of the photoaffinity label with high concentrations of i-dinoseb.

We conclude, therefore, that the binding protein responsible for inhibitory activity of phenolic herbicides is a protein of molecular weight approx. 41 000. This protein is probably part of the PS II reaction centre itself [18,25]. This result has to be compared with the results of Arntzen and co-workers [15–18] who have used the photoaffinity label azido-atrazine, an inhibitor which in its mode of action is similar to DCMU. In this case a protein of molecular weight approx. 32 000–34 000 is labelled.

It is of special interest to investigate in which way preincubation with other herbicides and inhibitors affects the chloroplast labelling pattern by the photoaffinity label. In Fig. 6 the labelling pattern of chloroplasts in the presence of increasing concentrations of DCMU is demonstrated (for convenience, only the part of the gel from 5 to 11 cm is shown). As indicated by the arrows, the amount of radioactivity found at increasing DCMU concentrations decreases only in the 41 kDa and 17 kDa proteins.

A similar experiment for a different kind of inhibitor, DNP-INT [26], an inhibitor at the reducing site of plastoquinone and in its mode of action similar to DBMIB, is depicted in Fig. 7. Increasing concentrations of DNP-INT do not decrease the amount of radioactivity found in either the 41 kDa or the 17 kDa protein. One should not be disturbed by the fact that the radioactivity found in the 41 kDa protein at 0.5 nmol DNP-INT is somewhat higher than that in the control. As already stressed this is due to the gel-slicing technique. In the experiment at 0.5 nmol DNP-INT, the radioactivity is probably completely centered within one 2-mm slice and the radioactivity amounts to a high level. In the other experiments, the radioactivity is divided into two adjacent slices and in addition radioactivity is lost in that portion of the gel where the knife cuts. This experiment, however, indicates that DNP-INT does not prevent the binding of the phenolic azide.

On the basis of our experimental results we present a model for the protein configuration at the reducing side of PS II as shown in Fig. 8. Two different proteins are responsible for inhibition of photosynthetic electron transport, a 41 kDa protein for inhibition by phenolic herbicides and a 32–34 kDa protein for the inhibition by DCMU and functionally related herbicides. Both proteins must be located close together and a conformational change of one, due to herbicide binding, influences the other. This has to be concluded for the following reasons:

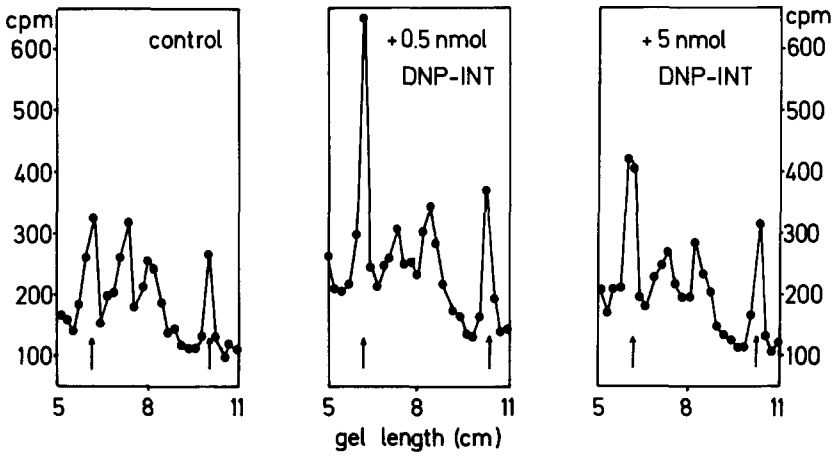

Fig. 7. Scan for radioactivity of a part of the SDS-polyacrylamide gels (11–15%) of chloroplasts labelled with 4-nitro-2-azido-6-[2',3'-^{3}H]isobutylphenol (8 nmol/mg Chl) in the presence of DNP-INT.

(i) Phenolic inhibitors can displace DCMU-like inhibitors [10] and vice versa, although the interference is non-competitive (Figs. 1, 2 and Ref. 19).

(ii) Occupation of the binding site of the 32–34 kDa protein by DCMU prevents the binding of the phenolic azide (Fig. 6).

In our model, we have placed the 32–34 kDa protein on top of the 41 kDa protein, the 32–34 kDa protein being located on the surface and accessible, the 41 kDa protein being non-accessible and hidden in the membrane. The arguments for this arrangement are the following:

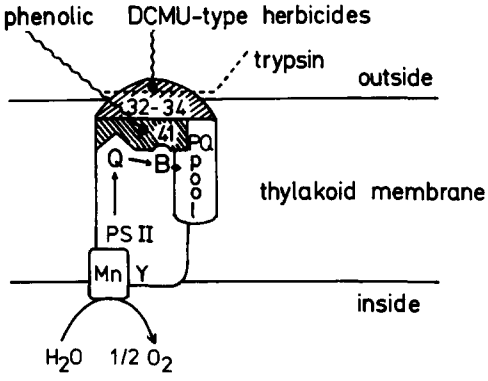

Fig. 8. Model for the location of two herbicide-binding proteins at the reducing side of Ps II. 32–34 and 41 represent (in kilodaltons) the corresponding proteins; PQ, plastoquinone.

(i) The 32–34 kDa protein, the 'proteinaceous shield' as proposed by Renger [27], can be digested by trypsin [22,23,27–32]. Trypsin cannot penetrate into the membrane. After trypsin treatment, DCMU and related herbicides can no longer bind specifically [22] and the DCMU sensitivity of photosynthetic ferricyanide reduction is lost to a large extent [22,23, 30].

(ii) Trypsin treatment does not affect, or only slightly so, the binding of the phenolic herbicides i-dinoseb [19], 2-iodo-4-nitro-6-isobutylphenol and ioxynil (Table I). Trypsin-treated chloroplasts in a short-term assay are even more sensitive towards phenolic herbicides in photosynthetic electron transport [31].

(iii) In PS II particles, the 32–34 kDa protein can be removed by mild detergent or trypsin treatment. This causes a loss of sensitivity in electron transport towards DCMU but not towards phenolic inhibitors [18,33].

(iv) Chloroplasts from atrazine-resistant mutants of *Amaranthus retroflexus* do not bind specifically any [^{14}C]atrazine [34] or the corresponding photoaffinity label [15,16]. Labelling of the 32–34 kDa protein is observed in the wild type but no longer in the mutant [15,16]. In contrast, chloroplasts from atrazine-resistant mutants show a 10-fold increased sensitivity towards phenolic herbicides in electron transport [35] and show no difference in phenolic herbicide binding compared to the wild type (Oett-

meier, W., Masson, K., Konze, H.J., and Fedtke, C., unpublished results).

(v) There exists a time lag before maximal inhibition in photosynthetic electron transport is achieved by phenolic herbicides [10]. This is in good agreement with a diffusion barrier for phenolic herbicides as exerted by the 32–34 kDa protein, until the phenolic herbicides have penetrated through to reach the underlying 41 kDa protein.

In this context, the role of the small 17 kDa peptide, which is also labelled by the phenolic azide and in which the amount of label is also diminished by incubation with i-dinoseb or DCMU, is not yet clear. In their investigation on the 'rapidly turning over' 32 kDa protein, Mattoo et al. [36] have also observed trypsin degradation products in this molecular weight range.

It has so far been speculated that the inhibition of photosynthetic electron transport by PS II herbicides may be due to lowering of the midpoint potential of a special plastoquinone bound to the B protein [2]. It might well be that inhibition of electron transport is a result of prevention of plastoquinone binding [14,37] to one or both of the 32–34 or 41 kDa proteins, thus leading to an interruption of the photosynthetic electron-transport chain.

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft. We are indebted to Professor Dr.A. Trebst for many stimulating discussions and to Dr. Doris Godde, Hildegard Berges and Hans-Joachim Soll for their help in some of the experiments.

References

1 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256
2 Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94
3 Trebst, A. and Draber, W. (1979) in Advances in Pesticide Science (Geissbühler, H., ed.), part 2, pp. 223–234, Pergamon Press, Oxford
4 Moreland, D.E. and Hilton, J.L. (1976) in Herbicides: Physiology, Biochemistry, Ecology (Audus, L.J., ed.), vol. 1, 2nd edn., pp. 493–523, Academic Press, New York
5 Moreland, D.E. and Blackmon, W.J. (1970) Weed Sci. 18, 419–426
6 Katoh, S. (1972) Plant Cell. Physiol. 13, 273–286
7 Van Rensen, J.J.S., Van der Vet, W. and Van Vliet, W.P.A. (1977) Photochem. Photobiol. 25, 579–583
8 Van Rensen, J.J.S., Wong, D. and Govindjee (1978) Z. Naturforsch. 33c, 413–420
9 Van Rensen, J.J.S. and Hobé, J.H. (1979) Z. Naturforsch. 34c, 1021–1023
10 Reimer, S., Link, K. and Trebst, A. (1979) Z. Naturforsch. 34c, 419–426
11 Trebst, A., Reimer, S., Draber, W. and Knops, H.J. (1979) Z. Naturforsch. 34c, 831–840
12 Oettmeier, W., Masson, K. and Johanningmeier, U. (1980) FEBS Lett. 118, 267–270
13 Oettmeier, W., Masson, K. and Johanningmeier, U. (1981) in Proceedings of the 5th International Congress on Photosynthesis, Halkidiki, Greece (Akoyunoglou, G., ed.), in the press
14 Oettmeier, W., Johanningmeier, U. and Trebst, A. (1981) in Quinones in Energy Conserving Systems (Trumpower, B.L., ed.), Academic Press, New York, in the press
15 Pfister, K., Steinback, K.E. and Arntzen, C.J. (1981) in Proceedings of the 5th International Congress on Photosynthesis, Halkidiki, Greece (Akoyunoglou, G., ed.), in the press
16 Pfister, K., Steinback, K.E., Gardner, G. and Arntzen, C.J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 981–985
17 Gardner, G. (1981) Science 211, 937–940
18 Mullet, J.E. and Arntzen, C.J. (1981) Biochim. Biophys. Acta 635, 236–248
19 Oettmeier, W. and Masson, K. (1980) Pestic. Biochem. Physiol. 14, 86–97
20 Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151
21 Tischer, W. and Strotmann, H. (1977) Biochim. Biophys. Acta 460, 113–125
22 Trebst, A. (1979) Z. Naturforsch. 34c, 986–991
23 Tischer, W. and Strotmann, H. (1979) Z. Naturforsch. 34c, 992–995
24 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
25 Satoh, K. (1979) Biochim. Biophys. Acta 546, 84–92
26 Trebst, A., Wietoska, H., Draber, W. and Knops, H.J. (1978) Z. Naturforsch. 33c, 919–927
27 Renger, G. (1976) Biochim. Biophys. Acta 440, 287–300
28 Regitz, G. and Ohad, I. (1976) J. Biol. Chem. 251, 247–252
29 Croze, E., Kelly, M. and Horton, P. (1979) FEBS Lett. 103, 22–26
30 Renger, G. (1979) Z. Naturforsch. 34c, 1010–1014
31 Böger, P. and Kunert, K.J. (1979) Z. Naturforsch. 34c, 1015–1020
32 Steinback, K.E., Pfister, K. and Arntzen, C.J. (1981) Z. Naturforsch. 36c, 98–108
33 Johanningmeier, U. (1981) Ph.D. Thesis, University of Bochum
34 Pfister, K., Radosevich, S.R. and Arntzen, C.J. (1979) Plant Physiol. 64, 995–999
35 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34c, 996–1009
36 Mattoo, A.K., Pick, U., Hoffman-Falk, H. and Edelman, M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 1572–1576
37 Velthuys, B.R. (1981) FEBS Lett. 126, 277–281